

CLAIMS

1. A fusion protein comprising at least three domains, where

5 - a first domain mediates membrane localization of the fusion protein in a cellular context, and
- a second domain has or presumably has a ligand-binding function of a nuclear receptor,
- a third domain has an activity able to activate a
10 signal pathway connected to a Ras protein in a cell, characterized in that when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain the third domain cannot exert its activity to activate a signal pathway connected to a
15 Ras protein in a cell, despite membrane localization.

2. A fusion protein as claimed in claim 1, characterized in that the individual domains are arranged within the fusion protein in the direction from the N terminus to the C terminus in the sequence first domain, second domain, third domain or in the sequence third domain, second domain, first domain.

3. A fusion protein as claimed in claim 1 or 2, characterized in that the inability of the third domain to activate a signal pathway connected to a Ras protein when there is a lack of binding of ligand to the second domain derives from the fact that when there is a lack of binding of ligand to the second domain the third domain can be complexed by a multiprotein complex attaching to the fusion protein in such a way that the third domain is unable to exert its activity to activate a signal pathway connected to a Ras protein in a cell.

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4. A fusion protein as claimed in claim 3, characterized in that it comprises an additional protein section within the second domain or as fourth domain for attachment of the multiprotein complex.

5. A fusion protein as claimed in any of claims 1 to
4, characterized in that the first domain comprises the
amino acid sequence of a membrane-localization signal,
5 in particular a farnesylation signal, myristylation
signal or prenylation signal or a transmembrane domain,
or is derived therefrom.

10 6. A fusion protein as claimed in any of the
preceding claims, characterized in that the amino acid
sequence of the second domain comprises an amino acid
sequence of a receptor section of a naturally occurring
nuclear receptor, or is derived therefrom.

15 7. A fusion protein as claimed in claim 6,
characterized in that the amino acid sequence of the
second domain comprises the amino acid sequence of the
receptor section of a steroid receptor, of an orphan
receptor, of a vitamin receptor, for example of a
20 vitamin D receptor, of a thyroxine receptor, of a
dioxin receptor or of a retinoic acid receptor, or is
derived therefrom.

25 8. A fusion protein as claimed in claim 6 or 7,
characterized in that the amino acid sequence of the
second domain comprises an amino acid sequence which is
derived from the amino acid sequence of a receptor
section of a naturally occurring nuclear receptor by
30 mutation, in particular by attachment, substitution,
deletion, insertion and/or modification of one or more
amino acids or groups of amino acids.

35 9. A fusion protein as claimed in any of claims 1 to
5, characterized in that the second domain is a non-
naturally occurring, synthetic receptor section which
is generated for example by "molecular modeling" and
has a ligand-binding function of a nuclear receptor.

10. A fusion protein as claimed in any of the preceding claims, characterized in that the third domain has the activity of an active and, in particular, constitutively active Ras protein.

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11. A fusion protein as claimed in any of claims 1 to 9, characterized in that the third domain has the activity of a functional guanine nucleotide exchange factor.

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12. A fusion protein as claimed in claim 11, characterized in that the amino acid sequence of the third domain is derived from the amino acid sequence of the CDC25 protein from *Saccharomyces cerevisiae*, of an SOS protein from a mammal or of an SOS-like protein from any organism.

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13. A fusion protein as claimed in claim 12, characterized in that the amino acid sequence of the third domain comprises at least the amino acid sequence sections of the CDC25 protein, of the SOS protein or of the SOS-like protein which are necessary for the activity of one of these proteins.

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14. A fusion protein as claimed in claim 1-11, characterized in that the third domain comprises an amino acid sequence which is derived from the amino acid sequence of a naturally occurring Ras protein or of a naturally occurring guanine nucleotide exchange factor or of the sections thereof necessary for the activity by mutation, and in particular by attachment, substitution, deletion, insertion and/or modification of one or more amino acids or groups of amino acids.

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15. A DNA molecule which encodes the fusion protein as claimed in any of claims 1 to 14.

16. A vector, in particular plasmid, cosmid, viral or phage genome, comprising at least one DNA molecule as claimed in claim 15.

5 17. A vector as claimed in claim 16, which is suitable for the transformation or transfection of a host cell.

10 18. A vector as claimed in claim 16, which is suitable for expression of at least one fusion protein, characterized in that it comprises at least one DNA molecule as claimed in claim 15 under the control of one or more promoters capable of functioning in a host cell.

15 19. A cell comprising a fusion protein as claimed in any of claims 1 to 14, characterized in that when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain of the fusion protein the third domain is unable to exert its activity to activate a signal pathway connected to a Ras protein in the cell, despite membrane localization, but when there is binding of ligand to the second domain or, in the alternative variant, when the ligand dissociates off from the second domain a conformational 20 change is brought about with effects on the third domain so that the third domain is able to exert its activity to activate a signal pathway connected to a Ras protein in the cell.

25 20. A cell as claimed in claim 19, characterized in that the inability of the third domain of the fusion protein to activate a signal pathway connected to a Ras protein when there is a lack of binding of ligand to the second domain of the fusion protein derives from the fact that when there is a lack of binding of ligand to the second domain the third domain can be complexed by a multiprotein complex attaching to the fusion protein in such a way that the third domain is unable 30 to exert its activity to activate a signal pathway

connected to a Ras protein in the cell, but when there is binding of ligand to the second domain a conformational change is brought about with effects on the third domain, resulting in the multiprotein complex 5 at least partly dissociating off from the fusion protein, and the third domain being able to exert its activity to activate a signal pathway connected to a Ras protein in the cell.

10 21. A cell as claimed in claim 19 or 20, characterized in that it comprises two or more fusion proteins as claimed in any of claims 1 to 14.

15 22. A cell as claimed in any of claims 19 to 21, characterized in that the cell is a single-cell prokaryotic or eukaryotic cell and, in particular, a yeast cell, specifically a yeast cell lacking cell walls.

20 23. A cell as claimed in any of claims 19 to 22, characterized in that in the absence of fusion protein at least under certain conditions a signal pathway connected to a Ras protein cannot be activated in the cell.

25 24. A cell as claimed in claim 23, characterized in that it comprises at least one fusion protein with a third domain which is able to activate the signal pathway connected to a Ras protein in the cell, which 30 is inactive or inactivatable in the absence of the fusion protein.

35 25. A cell as claimed in claim 23 or 24, characterized in that the signal pathway connected to a Ras protein acts on the cell cycle and its activation is essential for cell reproduction or the signal pathway connected to a Ras protein alternatively serves to activate transcription factors for genes which are not essential for cell reproduction.

26. A cell as claimed in any of claims 23 to 25, characterized in that the activatability of the signal pathway connected to a Ras protein is temperature-
5 dependent in the absence of fusion protein.

27. A cell as claimed in claim 26, characterized in that the lack of activatability of the signal pathway connected to a Ras protein in the absence of fusion
10 protein at particular temperatures is derived from at least one mutation of a guanine nucleotide exchange factor intrinsic to the cell, which has the effect that the latter is incapable of functioning above a particular temperature.

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28. A cell as claimed in claim 27, characterized in that it is a cell of the *Saccharomyces cerevisiae* yeast strain cdc25-2 or is derived from the latter.

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29. A cell as claimed in claim 27 or 28, characterized in that the cell comprises a fusion protein whose third domain has the activity of a functional guanine nucleotide exchange factor.

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30. A cell as claimed in claim 27 or 28, characterized in that the cell comprises a fusion protein whose third domain has the activity of an active and, in particular, constitutively active Ras protein.

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31. A cell as claimed in claim 26 or 27, characterized in that the lack of activatability of the signal pathway subsequent to a Ras protein in the absence of fusion protein at particular temperatures is derived from at least one mutation of a Ras protein intrinsic
35 to the cell, which has the effect that the latter is incapable of functioning above a particular temperature.

32. A cell as claimed in claim 23, characterized in that the lack of activatability of the signal pathway connected to a Ras protein in the absence of fusion protein derives from a deletion of the membrane-localization signal, in particular farnesylation signal, of the Ras protein intrinsic to the cell or from a mutation of this membrane-localization signal which has the effect that the Ras protein no longer binds to cellular membranes.

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33. A cell as claimed in claim 31 or 32, characterized in that the cell comprises a fusion protein whose third domain has the activity of an active and, in particular, constitutively active Ras protein.

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34. A cell as claimed in any of claims 19 to 33, characterized in that it is applied to a solid carrier.

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35. A cell as claimed in claim 34, characterized in that it is immobilized on biochips.

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36. An *in vivo* assay for determining the suitability of a test substance as ligand for a receptor section of a nuclear receptor, characterized by the following steps:

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(a) contacting the test substance with cells as claimed in any of claims 23 to 35 under conditions with which a signal pathway connected to a Ras protein cannot be activated in the cells in the absence of the fusion protein, where the fusion protein present in the cells contains a second domain comprising said receptor section, and a third domain which, when there is binding of ligand to the second domain, is able to activate the inactive signal pathway connected to a Ras protein,

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(b) investigating whether activation of the signal pathway connected to a Ras protein has taken place, where detection of the activation of the signal pathway connected to a Ras protein indicates the ability of the

test substance to bind to the second domain of the fusion protein and thus to the receptor section.

37. An assay as claimed in claim 36, where step (b) 5 comprises detecting the activation of the signal pathway connected to a Ras protein via reporter gene expression which takes place where appropriate and only because of the activation, resulting from the activation of the signal pathway connected to a Ras 10 protein, of a specific transcription factor, where detection of the expression of the reporter gene indicates the ability of the test substance to bind to the second domain of the fusion protein and, accordingly, to the receptor section.

15 38. An assay as claimed in claim 36, where in step (a) cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell 20 reproduction are employed, and step (b) comprises investigating whether the cells are capable of reproduction under said conditions, where detection of the ability of the cells to reproduce indicates the ability of the test substance to bind to the second 25 domain of the fusion protein and, accordingly, to the receptor section.

39. An *in vivo* assay for determining the suitability of a test substance as ligand for a receptor section of 30 a nuclear receptor, characterized by the following steps:

(a) contacting the test substance with cells as claimed in any of claims 23 to 35 under conditions with which a signal pathway connected to a Ras protein 35 cannot be activated in the cells in the absence of the fusion protein, where the fusion protein present in the cells contains a second domain comprising said receptor section, and a third domain which, only when there is binding of ligand to the second domain, is able to

activate the inactive signal pathway connected to a Ras protein,

(b) investigating whether activation of the signal pathway connected to a Ras protein has taken place,

5 (c) investigating cells employed in step (a) under conditions with which the signal pathway connected to a Ras protein in the cells cannot be activated in the absence of the fusion protein, for activation of the signal pathway connected to a Ras protein in the 10 absence of the test substance,

where detection of the activation of the signal pathway connected to a Ras protein in the absence of the test substance and the inactivity of the signal pathway connected to a Ras protein in the presence of the test 15 substance indicates the ability of the test substance to bind to the second domain of the fusion protein and thus to the receptor section.

40. An assay as claimed in any of claims 36 to 39, 20 characterized in that the test substance is a naturally occurring substance and, in particular, a hormone, in particular a steroid hormone, a vitamin, thyroxine or retinoic acid.

25 41. An assay as claimed in any of claims 36 to 39, characterized in that the test substance is a non-naturally occurring substance.

42. An assay as claimed in claim 41, characterized in 30 that the test substance is a synthetic derivative of a natural ligand or a poison, in particular dioxin.

43. A screening method for unknown ligands of a particular nuclear receptor, characterized in that an 35 assay method as claimed in any of claims 36 to 39 is employed for the screening.

44. An *in vivo* assay for detecting the presence of a ligand for a receptor section of a nuclear receptor in

a sample which possibly contains the latter, characterized by the following steps:

(a) contacting the sample with cells as claimed in any of claims 23-35 under conditions with which a signal 5 pathway connected to a Ras protein in the cell cannot be activated in the absence of the fusion protein, where the fusion protein comprises a second domain comprising said receptor section, and a third domain which is able to activate the signal pathway connected 10 to a Ras protein in the cells,

(b) investigating whether activation of the signal pathway connected to a Ras protein has taken place, where detection of the activation of the signal pathway connected to a Ras protein indicates the presence of a 15 ligand for the second domain of the fusion protein and thus for the receptor section of a nuclear receptor in the sample.

45. An assay as claimed in claim 44, where step (b) 20 comprises detecting the activation of the signal pathway connected to a Ras protein via reporter gene expression which takes place where appropriate and only because of the activation, resulting from the activation of the signal pathway connected to a Ras 25 protein, of a specific transcription factor, where detection of the expression of the reporter gene indicates the presence of a ligand for the second domain of the fusion protein and, accordingly, for the receptor section of a nuclear receptor in the sample.

30 46. An assay as claimed in claim 44, where in step (a) cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell 35 reproduction are employed, and step (b) comprises investigating whether the cells are capable of reproduction under said conditions, where detection of the ability of the cells to reproduce indicates the presence of a ligand for the second domain of the

fusion protein and, accordingly, for the receptor section of a nuclear receptor in the sample.

47. An in vivo assay for detecting the presence of a 5 ligand for a receptor section of a nuclear receptor in a sample which possibly contains the latter, characterized by the following steps:

(a) contacting the sample with cells as claimed in any 10 of claims 23-35 under conditions with which the signal pathway connected to a Ras protein in the cell cannot be activated in the absence of fusion protein, where the fusion protein comprises a second domain comprising said receptor section, and a third domain which is able to activate the inactive signal pathway connected to a 15 Ras protein in the cells,

(b) investigating whether an activation of the signal pathway connected to a Ras protein has taken place,

(c) investigating cells employed in step (a) under 20 conditions with which a signal pathway connected to a Ras protein in the cells cannot be activated in the absence of fusion protein, for activation of the signal pathway connected to a Ras protein in the absence of the sample, where a detection of the activation of the signal pathway connected to a Ras protein in the 25 absence of the sample and the inactivity of the signal pathway connected to a Ras protein in the presence of the sample indicates the presence of a ligand for the second domain of the fusion protein and thus for the receptor section of a nuclear receptor in the sample.

30 48. A screening method for unknown ligands of a particular nuclear receptor in a sample, characterized in that an assay method as claimed in any of claims 44 to 47 is employed for the screening.

35 49. An in vivo assay for the quantitative determination of the concentration of a ligand for the receptor section of a nuclear receptor in a sample

which contains the latter, characterized by the following steps:

(a) contacting an aliquot of the sample with cells as claimed in any of claims 23 to 35 under conditions with 5 which a signal pathway connected to a Ras protein in the cell cannot be activated in the absence of the fusion protein, where the fusion protein present in the cells comprises said receptor section, and contains a second domain comprising said receptor section, and a third domain which is able to activate the inactive 10 signal pathway connected to a Ras protein in the cells,

(b) detecting quantitatively the extent of the activation of the signal pathway connected to a Ras protein by direct or indirect means,

15 (c) measuring the concentration of the ligand in the sample by comparing the measured extent of activation with corresponding values measured for known standard concentrations of the ligand.

20 50. An assay as claimed in claim 49, characterized in that the quantitative detection of the extent of activation of the signal pathway connected to a Ras protein in step (b) takes place indirectly by determining the amount present in the cells of a 25 transcription or translation product of a reporter gene whose expression takes place only because of the activation, resulting from the activation of the signal pathway connected to a Ras protein, of a specific transcription factor, at a particular time or the 30 expression rate of this reporter gene based on the transcription or translation product under said conditions, and in step (c) the measurement of the concentration of the ligand in the sample takes place by comparing the measured values with corresponding 35 values measured for known standard concentrations of the ligand.

51. An assay as claimed in claim 49, characterized in that in step (a) cells in which the inactive signal

pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell reproduction are employed, and the quantitative detection of the extent of the activation 5 of the signal pathway connected to a Ras protein in step (b) takes place indirectly by determining the reproduction of the cells at a fixed time or the reproduction rate of the cells under said conditions, and in step (c) the measurement of the concentration of 10 the ligand in the sample takes place by comparing the measured values with corresponding values measured for known standard concentrations of the ligand.

52. An *in vivo* assay for detecting whether a compound 15 is able to alter a binding activity of a receptor section of a nuclear receptor in relation to a ligand, characterized by the following steps:

(a) contacting the ligand in the presence of the compound with cells as claimed in any of claims 23 to 20 35 under conditions with which the compound can diffuse into the cells or it is produced by the cells, and with which in the absence of fusion protein a signal pathway connected to a Ras protein in the cells cannot be activated, where the fusion protein present in the 25 cells comprises a second domain comprising said receptor section, and a third domain which is able to activate the inactive signal pathway connected to a Ras protein in the cells only when there is binding of the ligand or, alternatively, only when there is lack of 30 binding of ligand to the second domain,

(b) investigating whether and, where appropriate, to what extent activation of the signal pathway connected to a Ras protein takes place,

35 (c) comparing the result of the investigation in step (b) with a result of an investigation obtained when the assay is carried out in the absence of the compound.

53. An assay as claimed in claim 52, characterized in that step (b) comprises detecting the activation of the signal pathway connected to a Ras protein via reporter gene expression which takes place where appropriate and only because of the activation, resulting from the activation of the signal pathway connected to a Ras protein, of a specific transcription factor, and the quantitative detection, which takes place where appropriate, of the extent of the activation of the signal pathway connected to a Ras protein comprises determining the amount, present in the cells, of transcription or translation product of the reporter gene at a particular time or the expression rate of this reporter gene based on the transcription or 10 translation product under said conditions, and in the case where the comparison in step (c) reveals that stronger expression of the reporter gene occurs in the presence of the compound, an agonistic effect of the compound is indicated, and in the case where the comparison in (c) reveals that lower expression of the reporter gene occurs in the presence of the compound, 15 an antagonistic effect of the compound is indicated.

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54. An assay as claimed in claim 53, characterized in that it is carried out under conditions with which no reproduction of the cells occurs.

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55. An assay as claimed in claim 52, where in step (a) there is use of cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is essential for cell reproduction, and step (b) comprises investigating whether and, where appropriate to what extent, the cells are able to reproduce under said 30 conditions, and in the case where the comparison in step (c) reveals that greater cell reproduction occurs in the presence of the compound, an agonistic effect of the compound is indicated, and in the case where the comparison in step (c) reveals that less cell 35

reproduction occurs in the presence of the compound, an antagonistic effect of the compound is indicated.

56. An *in vivo* assay for detecting whether a
5 polypeptide or protein has a ligand-binding function of
a nuclear receptor, characterized by the following
steps:

(a) contacting cells as claimed in any of claims 23 to
10 35 with the ligand under conditions with which a signal
pathway connected to a Ras protein cannot be activated
in the cells in the absence of the fusion protein,
where the fusion protein present in the cells comprises
15 a second domain which comprises the polypeptide or
protein to be investigated, and a third domain which is
able to activate the inactive signal pathway connected
to a Ras protein in the cells when there is binding of
ligand to the second domain,

(b) investigating whether an activation of the signal
pathway connected to a Ras protein has taken place,
20 where detection of the activation of the signal pathway
connected to a Ras protein indicates that the second
domain of the fusion protein and, accordingly, the
polypeptide or protein to be investigated has a ligand-
binding function of a nuclear receptor.

25 57. An assay method as claimed in claim 56,
characterized in that the fusion protein present in the
cells comprises a second domain which contains a
30 receptor section derived from a naturally occurring
receptor section by mutation.

58. An assay as claimed in claim 56 or 57, where step
(b) comprises detecting the activation of the signal
pathway connected to a Ras protein via reporter gene
35 expression which takes place where appropriate and only
because of the activation, resulting from the
activation of the signal pathway connected to a Ras
protein, of a specific transcription factor, where
detection of the expression of the reporter gene

indicates the presence of a ligand-binding function of the second domain of the fusion protein and, accordingly, of the polypeptide or protein to be investigated.

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59. An assay as claimed in claim 56 or 57, where in step (a) cells in which the inactive signal pathway connected to a Ras protein is a signal pathway which acts on the cell cycle and whose activation is 10 essential for cell reproduction are employed, and step (b) comprises investigating whether the cells are capable of reproduction under said conditions, where detection of the ability of the cells to reproduce indicates the presence of a ligand-binding function of 15 the second domain of the fusion protein and, accordingly, of the polypeptide or protein to be investigated.

60. An *in vivo* assay for detecting whether a 20 polypeptide or protein has a ligand-binding function of a nuclear receptor, characterized by the following steps:

(a) contacting cells as claimed in any of claims 23 to 25 35 with the ligand under conditions with which a signal pathway connected to a Ras protein in the cells cannot be activated in the absence of the fusion protein, where the fusion protein present in the cells comprises a second domain which comprises the polypeptide or protein to be investigated, and a third domain which is 30 able to activate the inactive signal pathway connected to a Ras protein in the cells only when there is a lack of binding of ligand to the second domain,

(b) investigating whether an activation of the signal pathway connected to a Ras protein has taken place,

35 (c) investigating cells as employed in step (a) under conditions with which a signal pathway connected to a Ras protein cannot be activated in the cells in the absence of the fusion protein, for activation of the

signal pathway connected to a Ras protein in the absence of ligand,

5 where a detection of the activation of the signal pathway connected to a Ras protein in the absence of the ligand and the inactivity of the signal pathway connected to a Ras protein in the presence of the ligand indicates that the second domain of the fusion protein and, accordingly, the polypeptide or protein to be investigated has a nuclear receptor.

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61. A kit for use in an assay or screening method as claimed in any of claims 36 to 55, characterized in that it comprises cells as claimed in claim 23.

15 62. A kit for use in an assay as claimed in any of claims 36 to 55, characterized in that it comprises the following constituents:

a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,

b) one or more transformation or transfection vectors which contain at least one DNA sequence which encodes a fusion protein as claimed in claim 1, where the fusion protein comprises a third domain which is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is lack of binding, or alternatively, when there is binding of ligand to the second domain,

c) where appropriate reagents for transformation or transfection of the cells with the transformation or transfection vector,

d) where appropriate reagents for detecting the phenotypical activation of the signal pathway connected to a Ras protein in these cells.

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63. A kit for use in an assay as claimed in any of claims 36 to 55, characterized in that it comprises the following constituents:

a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,

5 b) a transformation or transfection vector which has, in suitable arrangement,

- a DNA sequence which encodes a first domain of a fusion protein as defined in claim 1,
- a DNA sequence which encodes a third domain of a fusion protein as defined in claim 1 and which is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain, and
- 10 -- a suitably arranged insertion site for functional insertion of a DNA sequence which encodes a second domain as defined in claim 1, where, after insertion of a DNA sequence for the second domain, the vector comprises a complete gene for a fusion protein as claimed in claim 1,
- 15 c) where appropriate reagents for transformation or transfection of the cells with the transformation or transfection vector,
- d) where appropriate reagents for detecting the phenotypical activation of the signal transduction pathway connected to a Ras protein in these cells.

64. A kit for use in an assay as claimed in any of claims 56 to 60, characterized in that it comprises cells as claimed in claim 23, where the fusion protein present therein comprises a second domain comprising a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor.

35 65. A kit for use in an assay as claimed in any of claims 56 to 60, characterized in that it comprises the following constituents:

- a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,
- 5 b) one or more transformation or transfection vectors which comprise at least one DNA sequence which encodes a fusion protein as claimed in claim 1, whose second domain comprises a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor, and whose third domain is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain,
- 10 c) where appropriate reagents for transformation or transfection of the cells with the transformation or transfection vector,
- d) where appropriate reagents for detecting the phenotypical activation of the signal transduction pathway connected to a Ras protein in these cells.

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66. A kit for use in an assay as claimed in any of claims 56 to 60, characterized in that it comprises the following constituents:

- a) cells in which at least under certain conditions a signal pathway connected to a Ras protein cannot be activated,
- 25 b) a transformation or transfection vector which has, in suitable arrangement,
 - a DNA sequence which encodes a first domain of a fusion protein as defined in claim 1, and
 - a DNA sequence which encodes a third domain of a fusion protein as defined in claim 1 and which is able to activate the inactive or inactivatable signal pathway connected to a Ras protein in the cells when there is a lack of binding or, alternatively, when there is binding of ligand to the second domain, and
 - a suitably arranged insertion site for functional insertion of a DNA sequence which

encodes a second domain comprising a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor,
where, after insertion of a DNA sequence for the second
5 domain, the vector comprises a complete gene for a fusion protein as claimed in claim 1, where the second domain comprises a polypeptide or protein suspected of having a ligand-binding function of a nuclear receptor,
c) where appropriate reagents for transformation or
10 transfection of the cells with the transformation or transfection vector,
d) where appropriate reagents for detecting the phenotypical activation of the signal pathway connected to a Ras protein in these cells.

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67. A kit as claimed in any of claims 61 to 66, in which the cells additionally contain a construct comprising a binding site for a transcription factor whose activation results from an activation of a
20 specific ras signal pathway whose activation is to be detected by the assay, a minimal promoter and a reporter gene functionally linked thereto, where the minimal promoter is activated as a result of binding of the activated transcription factor to its binding site.

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68. A kit as claimed in any of claims 61 to 66, characterized in that it additionally contains a transformation or transfection vector with a construct comprising a binding site for a transcription factor whose activation results from an activation of a
30 specific ras signal pathway whose activation is to be detected by the assay, a minimal promoter and a reporter gene functionally linked thereto, where the minimal promoter is activated as a result of a binding
35 of the activated transcription factor to its binding site.

69. A kit as claimed in any of claims 61 to 66, characterized in that it additionally contains a

transformation or transfection vector with a construct comprising a binding site for a transcription factor whose activation results from an activation of a specific ras signal pathway whose activation is to be 5 detected by the assay, a minimal promoter and an insertion site, suitably arranged for expression controlled by the minimal promoter, for insertion of a gene for a reporter protein, where the minimal promoter is activated as a result of a binding of the activated 10 transcription factor to its binding site.

70. A kit as claimed in any of claims 61 to 69, which contains the cells immobilized on a solid carrier, in particular on microtiter plates or biochips.

15 71. A ligand for a binding section of a receptor, compound which is able to alter a binding activity of a ligand-binding section of a receptor in relation to a ligand, or polypeptide or protein which has a ligand- 20 binding function of a receptor, obtained or identified by means of one of the assay methods as claimed in any of claims 36 to 48 and 52 to 60.

25 72. A composition which contains one or more ligand(s), one or more compound(s) and/or one or more polypeptide(s) or protein(s) as claimed in claim 71.

30 73. A pharmaceutical comprising a ligand, a compound and/or a polypeptide or protein as claimed in claim 71 or a composition as claimed in claim 72.

74. A method for identifying polypeptides or proteins, in particular receptors, which have a ligand-binding function of a receptor, which comprises:
35 - preparing a cell as claimed in claim 1 with a fusion protein having the features described in claim 1 and comprising the whole of such a polypeptide or protein or a part of such a polypeptide or protein which presumably contains

the sequence sections essential for the ligand-binding function, and

5 using this cell to carry out an *in vivo* assay method for detecting whether a polypeptide or protein has a ligand-binding function of a receptor, as claimed in any of claims 56 to 60.

10 75. The use of ligand, of a compound and/or of a polypeptide or protein as claimed in claim 71 as lead substance for developing ligands, compounds and polypeptides or proteins derived therefrom.

15 76. A method for preparing a ligand for a binding section of a receptor, a compound which is able to alter a binding activity of a ligand-binding section of a receptor in relation to a ligand, or a polypeptide or protein which has a ligand-binding function of a receptor, by derivatization one or more times starting from a ligand, modifying compound, polypeptide or 20 protein identified by the assay, identification, screening or preparation methods as claimed in any of claims 36 to 48, 52 to 60 and 74.

25 77. A nucleic acid molecule obtained starting from a polypeptide or peptide, in particular receptor, identified or prepared by the assay or preparation methods as claimed in any of claims 56 to 60, 74 and 76, by a method which comprises the provision of a gene encoding the polypeptide or protein, or a part, which 30 comprises at least the nucleic acid sequence sections essential for the activity of the encoded polypeptide or protein, in essentially pure form.

35 78. The use of a nucleic acid molecule as claimed in claim 77 for preparing a gene therapeutic agent.